USER GUIDE

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PureLink® HQ Mini Plasmid Purification Kit

For isolating high-quality plasmid DNA

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Contents

Kit Contents and Storage 4

Introduction 5

Methods 6

Purification Procedure 6

Plasmid DNA Yield and Quality 9

Troubleshooting 10

Technical Support 12

Kit Contents and Storage

Shipping and Storage

All components of the PureLink® HQ Mini Plasmid Purification Kit are shipped at room temperature. Upon receipt, store all components at room temperature, with the following exception:

- After addition of RNase, the Resuspension Buffer should be stored at 4°C.

Contents

The components included in the PureLink® HQ Mini Plasmid Purification Kit are listed in the following table. Sufficient reagents are provided in the kit to perform 100 isolations.

Component	Amount
Resuspension Buffer	120 mL
Lysis Buffer	120 mL
Neutralization/Binding Buffer	2 × 85 mL
Wash Buffer	16 mL
Elution Buffer (10 mM Tris-HCl, pH 8.5)	15 mL
RNase A	12 mg
Spin Columns	100 each
Collection Tubes (2 mL)	100 each
Elution Tubes (1.7 mL)	100 each

Introduction

Introduction

The PureLink® HQ Mini Plasmid Purification Kit is designed for the isolation of high-quality plasmid DNA that is suitable for restriction enzyme digestion, PCR, sequencing, bacterial cell transformation, and mammalian cell transfection.

Using the kit, plasmid DNA can be isolated from varying amounts of bacterial cells. The high quality of the isolated plasmid DNA is demonstrated by its low genomic DNA contamination, high supercoiled to nicked forms ratio, and reliable performance in demanding downstream applications such as mammalian cell transfection.

System Overview

Bacterial cells are pelleted, resuspended, and lysed. The lysate is then neutralized and conditions are adjusted for subsequent binding. After clarification by centrifugation, the lysate is processed through the PureLink® spin column. The DNA binds to the silica-based membrane in the column, and impurities are removed by a single wash step. The DNA is then eluted in Elution Buffer or water.

Advantages

The PureLink® HQ Mini Plasmid Purification Kit has the following advantages:

- Higher spin column capacity for plasmid DNA as compared to other commercially available plasmid purification systems
- Designed to isolate high-quality plasmid DNA in less than an hour
- Minimal genomic DNA contamination of the purified sample
- Reliable performance of the purified DNA in downstream applications

Methods

Purification Procedure

Materials Supplied by the User

- 64 mL 100% ethanol
- Sterile microcentrifuge tubes
- Tabletop microcentrifuge

Before Starting

- Prepare the Resuspension Solution with the RNase A supplied in the kit. Resuspend the lyophilized RNase A (12 mg) in 200 µL of Resuspension Solution, and then add the resuspended mixture to the remaining Resuspension Solution for a final concentration of 0.1 mg/mL RNase A.

  After mixing, store the Resuspension Solution with RNase A at 4°C. Stable for up to 6 months.
- Prepare the Wash Buffer with ethanol. Add 64 mL of 96–100% ethanol to the entire volume of Wash Buffer (16 mL).
- Before each use, check the Neutralization/Binding Buffer and Lysis Buffer for a white salt precipitate. If present, place each buffer in a 37°C water bath for 5 minutes or until the salts redissolve and the solution clears.

  Do not shake the Lysis Buffer, as this can lead to foaming.

Purification Procedure, Continued

Column Capacity

Each column has a DNA binding capacity of up to 60 µg plasmid DNA.

For cell volumes >2×10^9 cells, prepare cells as separate lysates of ≤$1–2 \times 10^9$ cells each as described below, and load lysates consecutively on the same column as described in **Binding DNA**, page 8, Step 3.

Preparing Bacterial Cell Lysates

To prepare the bacterial cell lysate:

1. In a microcentrifuge tube (not supplied in the kit), pellet 1–3 mL ($1–2 \times 10^9$) of *E. coli* cells from overnight cultures by centrifugation in a tabletop centrifuge at $1500 \times g$ for 15 minutes. Remove the culture media completely.
2. Completely resuspend the pellet in 240 µL of Resuspension Solution, prepared with RNase A as described in **Before Starting**.
3. Add 240 µL of Lysis Buffer to the above solution. Mix gently by inverting the tube 4–8 times.
4. Incubate for 3–5 minutes at room temperature. Do not exceed 5 minutes.
5. Add 340 µL of Neutralization/Binding Buffer, and immediately mix gently by inverting the tube 4–8 times.
6. Centrifuge for 10 minutes at maximum speed in a tabletop centrifuge to clarify the cell lysate.

Proceed to **Binding DNA**.

Purification Procedure, Continued

Binding DNA

1. Place a PureLink® spin column inside a 2-mL collection tube.
2. Pipette or decant the supernatant from step 6, **Preparing Bacterial Cell Lysates**, into the spin column.
3. Centrifuge the column at room temperature at 10,000–14,000 × *g* for 1 minute. Discard the flowthrough, and place the column back in the tube.

   If you are loading multiple samples on the same column, repeat Steps 2–3 for each lysate preparation.

   If you are using an endA+ *E. coli* strain, we recommend adding an additional wash step before proceeding to the next step. See page 11.
4. Add 650 µL of Wash Buffer, prepared with ethanol as described on page 6, to the column.
5. Centrifuge the column at room temperature at 10,000–14,000 × *g* for 1 minute. Discard the flowthrough from the collection tube, and place the column back in the tube.
6. Centrifuge the column at maximum speed for 1–3 minutes to remove the residual wash buffer.
7. Proceed to **Eluting DNA**.

Eluting DNA

1. Place the spin column in a clean 1.7-mL elution tube.
2. Add the following volume of Elution Buffer (10 mM Tris-HCl, pH 8.5) or sterile, distilled water (pH >7.0):
   - Add 50 µL of Elution Buffer or water to the center of the column if the expected DNA yield is <30 µg.
   - Add 100 µL of Elution Buffer or water to the center of the column if the expected DNA yield is >30 µg.
3. Incubate the column at room temperature for 1 minute, then centrifuge at maximum speed for 1 minute.
4. The elution tube contains your purified DNA. Remove and discard the column.

Determine the quantity and quality of the DNA as described in **Plasmid DNA Yield and Quantity**.

Plasmid DNA Yield and Quality

Note

Plasmid DNA yield will vary depending on a variety of factors, including:

- Number of plasmid copies per cell
- Plasmid size
- Size of the insert
- Effect of the insert on plasmid propagation
- Bacterial culture growth

Estimating DNA Yield

You can estimate the quantity of the purified plasmid DNA with a spectrophotometer using UV absorbance at 260 nm as follows:

1. Dilute an aliquot of the plasmid DNA sample in 10 mM Tris-HCl, pH 7.5. Mix well. Transfer to a cuvette (1-cm optical path length).
2. Measure the A_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.
3. Calculate the amount of DNA using the following formula:

DNA (µg) = A_{260} × 50 µg/(1 A_{260} × 1 mL) × dilution factor × total sample volume (mL)

Assumption: For dsDNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Estimating DNA Quality

Typically, DNA isolated using the PureLink® HQ Mini Plasmid Purification Kit has an A_{260}/A_{280} >1.80 when samples are diluted in Tris-HCl (pH 7.5). An A_{260}/A_{280} of >1.80 indicates that the DNA is reasonably clean of proteins that could interfere with downstream applications.

Absence of contaminating genomic DNA and RNA may be confirmed by agarose gel electrophoresis.

Troubleshooting

Problem	Possible Solution
Low yield	• Check the growth conditions of the cell culture to ensure plasmid propagation. Use a high copy number plasmid if possible. • For low copy number plasmids, increase the amount of cell culture used to 4–6 mL and process as three separate samples. • If the cell lysate is too viscous, reduce the amount of cells used per sample. • Ensure complete resuspension of the bacterial cell pellet. • Ensure that the DNA elution was performed with a low salt buffer (e.g., the Elution Buffer supplied in the kit) or sterile water (pH >7.0)
Plasmid DNA degradation	• Avoid using endA+ bacterial strains if possible. • If you are using an endA+ strain, we recommend adding an additional wash step before Step 4, Binding DNA, using 500 µL of 40% isopropanol, 4.0 M guanidine-HCl (pH 6.9–7.1). See the preparation instructions on the following page.
Genomic DNA contamination in the eluate	• Perform Steps 3–5, **Preparing Cell Lysate**, with gentle mixing to avoid shearing genomic DNA. Genomic DNA must be intact to be efficiently precipitated away from the plasmid DNA. • Do not exceed 5 minutes of cell lysis, as stated in the protocol (Step 4, **Preparing Cell Lysate**).

Optional Wash Step

If you are using an endA+ bacterial strain, we recommend adding an additional wash step after Step 3, **Binding DNA** (page 8), using 500 µL of 40% isopropanol, 4.0 M guanidine-HCl (pH 6.9–7.1). To prepare a 50-mL solution:

1. Prepare a 7.5 M guanidine hydrochloride (GuHCl) solution by dissolving GuHCl (UltraPure™ GuHCl, Cat. no. 15502-016), 71.5 g in 70 mL sterile water.
2. Heat solution at 37°C with constant stirring to facilitate dissolving.
3. Bring to a volume of 100 mL with sterile water in a volumetric flask.
4. Mix 27 mL of the solution with 20 mL of isopropanol.
5. Bring to a volume of 50 mL with sterile water in a volumetric flask. Mix thoroughly by inverting. The pH will be 6.9–7.1 without further adjustment.
6. Immediately after Step 3, **Binding DNA** (page 8), add 500 µL of the guanidine-HCl/isopropanol solution from the previous step to the column, and centrifuge at room temperature at 10,000–14,000 × *g* for 1 minute. Discard the flowthrough. Then proceed to Step 4, **Binding DNA**.

Technical Support

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Technical Support, Continued

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